1959

HOSPITAL FOR SPECIAL SURGERY

96th Annual Report

1959

REPORT OF THE DIRECTOR OF RESEARCH

Report of the Director of Research

Alfred H. Caspary Research Building

This is the official name of the new building, now nearly completed on the north side of 71st Street and facing the East River, which will be joined with the Hospital by a bridge. This building is to be occupied by the Philip D. Wilson Research Foundation, which has been organized to serve as the research arm of the Hospital for Special Surgery. It exists chiefly as an administrative and financial entity with responsibility for controlling the finances and activities in the research laboratories of the new building. It is considered undesirable to make a separation between the research activities carried on here and those in the hospital. In fact, all appointments are of a dual nature, and staff members of the Foundation are considered to be staff members of the hospital, and vice versa. Publications from either the hospital or the Foundation, will bear the acknowledgement "From the Hospital for Special Surgery-Philip D. Wilson Research Foundation, affiliated with the Cornell University Medical College."

Construction of a new building, is always fraught with delays, which to the expectant owners seems interminable. This seemed true in our case, but now looking back, we realize that no major obstacles were encountered, and that on the whole, we were fortunate. We began moving the research laboratories and personnel from the hospital to the new building in October, and little by little over the course of the next two or three months, the laboratories were completed and occupied. Although we had done a lot of careful planning, occupation of the laboratories revealed some deficiencies, the major one being too little electrical power. Extra expenditures and additional work were required to rectify these mistakes. Some of them were caused by changes in requirements since the original planning was done in 1956, and the appointment of additional investigators to the staff. We now have a satisfactory set up and the work is proceeding smoothly. On the whole, our investigators are delighted with their new laboratories and with their splendid equipment and the convenient services that are provided. Certainly, with the wonderful outlook upon the East River, these laboratories will rank with the most beautiful to be found anywhere.

The Caspary building provides five floors and an automobile parking area underneath, capable of holding approximately 40 cars. The elevators have been extended upwards to provide service for two additional floors, which can be readily added, in case they should ever be needed. Each of these abbreviated floors carries a penthouse area of about 2400 square feet, which can be used for additional research space if needed in

the meantime. The first and second floors have areas each of approximately 10,000 square feet and each of the other three floors have approximately 8,000 square feet. The second floor is being reserved for future unspecified hospital purposes. This is in view of the consideration that present hospital space is fully occupied and that there may be need for expansion at some future time. The fifth floor is at present vacant, but is earmarked for future enlargement of research activities when necessary.

We have presently completed and equipped the first, third and fourth floors. These have been developed wholly for laboratory and experimental research and no facilities for patient care have been provided. The fourth floor is occupied by laboratories for biological chemistry and there is also a Conference Room, which will accommodate about fifty persons. We have, on this floor, two laboratories for the study of mineral metabolism, under Dr. Felix Bronner, three laboratories for analytical biochemistry, under Dr. C. J. Umberger, two laboratories for the study of Collagen Physiology and Chemistry, one under Drs. Robert Watson and Sidney Rothbard, and one under Dr. William Robbins. There is also large laboratory and associated facilities occupied by Dr. Ralph Heimer. He is studying the Macroproteins produced in rheumatoid arthritis and the connective tissue diseases. There are two offices, separate rooms for extraction and digestive procedures, and a glassware wash-up room. There are, in addition, four walk-in thermo regulated rooms which are furnished with control arrangements for maintaining either constant temperatures at different levels or low humidity.

The third floor is set aside for research in Pathology, Microbiology and Immunology including the study of ultra structure with the electron microscope. It was designed by Dr. Robert C. Mellors, Pathologist to the Hospital and Associate Director of Research, to provide facilities for coordinated research in cancer, the rheumatic diseases and other crippling conditions, in association with other investigators. We have been fortunate in obtaining the services of Dr. Leonhard Korngold, Immunologist, Dr. Fred Rapp, Microbiologist and Dr. James C. Harkin, Assistant Pathologist to the Hospital and Electron microscopist. The floor provides laboratories for experimental pathology, for histochemistry, for electronmiscroscopy, for immunology and microbiology, and for biochemistry. There is also a laboratory for fluorescent microscopy. There are various office areas and instrument room, and a walk-in thermo-regulated cold room.

The first floor contains the animal population, as well as the offices for the administrative staff of the Foundation. One room is set aside for experiments using radio-active isotopes, two rooms for the reception and quarantine of all new additions to the animal population. Isolation facilities are also provided for work on animals which may involve the use of infective agents that might be harmful to the other animals serving the Foundation unless carefully controlled. A large cyclic type of cage washer, using detergents and live steam for cleaning, has been installed which will handle whole racks of animal cages. A large high pressure sterilizer is being added to decontaminate cages that have been used to contain animals harboring live infective agents.

Small areas on the second floor have been excluded from the hospital space to provide stockroom and also facilities for Scoliosis research.

RESEARCH PROJECTS

A. Rheumatic Disease Section

1-50-R Richard H. Freyberg, M.D., Director

Clinical Studies

A variety of different projects chiefly of clinical investigational nature have been continued under this grant; a few new researches have been started. The comprehensive review of experiences of this clinic for rheumatic diseases and personal experiences in office practice of senior staff members, is being summarized by Drs. R. Cecil and W. Kammerer particularly to compare results of several special measures of treatment for rheumatoid arthritis, especially gold therapy and the corticosteroids. This has been progressing for three years and may require another year for completion.

For two years Dr. Carl Berntsen has been making a study of the status of rheumatoid arthritic patients after five or more years of treatment with corticosteroids. The results of study of 168 patients were presented by Dr. Berntsen at the scientific session of the Second Pan American Congress on Rheumatic Diseases held in Bethesda, June 1959. This study is being continued and expanded and results in 183 patients will form the report Dr. Berntsen will make at the meeting of the American College of Physicians in San Francisco, April 1960.

Dr. Bernard Rogoff has completed a three year study of pathologic and clinical aspects of progressive systemic sclerosis (scleroderma) and the effects of releasin as a therapeutic agent for this diffuse connective tissue disease.

Ulcerogenic effects of corticosteroids used in treatment of patients with rheumatoid arthritis has been the continued interest of Dr. W. Kammerer, who reported results of his studies before the Second Pan American Congress on Rheumatic Diseases in Bethesda, June 1959.

The effects of local infiltration of a solution of ammonium sulfate about the painful joints of patients with osteoarthritis is being studied by Dr. M. Wald. Another preparation, proposed as oral systemic treatment to relieve pain and stiffness of osteoarthritis — certain lipoid fractions of soy bean — is being evaluated (using the controlled double-blind technique) by Dr. George Ehrlich.

Dr. J. Gascon has completed his study of the factors influencing the development of osteoporosis and pathologic fractures of osteoporotic bone in rheumatoid patients who received prolonged corticosteroid therapy. Results of this study were presented at a recent meeting of the Montreal Medical and Chirurgical Society.

The comparison of intra-articularly injected suspensions of corticosteroids has been continued with the aid of Dr. G. Ehrlich. This past year, two newly prepared suspensions have been added to those being evaluated. This study continues.

Collaborating with members of the Department of Dermatology of C.U.M.C., Dr. H. Wainerdi has been pursuing a systemic clinical study which is planned to help answer the question of whether localized dermatitis or lupus erythematosis, ever become

systemic lupus erythematosis, and if so how frequently and under what circumstances. The completion of this study is expected to take five years or longer.

A new project has been started during 1959 by Dr. G. Ehrlich — a review of patients and records to determine the incidence, type, and clinical importance of cardiovascular pathology considered to be a part of rheumatoid disease.

The laboratory studies which form a part of some of these clinical investigations will be separately reported on by Dr. R. Heimer, chief of the rheumatic disease research laboratories.

Research Fellows in Rheumatic Disease who completed their year's appointment June 30, 1959 are Dr. J. Gascon, who has returned to Montreal, Canada, and Dr. D. Sabah, who has again taken up academic medical pursuits in Chile. Dr. George Ehrlich, a trainee of the Public Health Service, joined our staff as Research Fellow in Rheumatic Diseases, and Instructor in Medicine, C.U.M.C., July 1st, 1959.

Acknowledgement is again gratefully made to the National Institute of Arthritis and Metabolic Diseases, the New York Chapter of the Arthritis and Rheumatism Foundation, the Lederle Laboratories, Inc., and several individual (anonymous) donors, whose grants-in-aid for research in rheumatic disease to this institution have made possible many of these investigations.

Rheumatic Disease Laboratory — Dr. Ralph Heimer

(1) *Rheumatoid Factor*

(a) ISOLATION AND CHARACTERIZATION:

Work continued on isolation of rheumatoid factor from individual serum samples of patients with Rheumatoid Arthritis. Various techniques, developed previously in the laboratory, were used. The results indicated that a number of proteins having the characteristics of a macroglobulin could be isolated from a single serum sample.

Work on the characterization of the rheumatoid factors was further pursued. The rate of sedimentation of representative samples as well as their molecular weight, size and shape was studied.

A number of purified preparations were subjected to total amino acid analyses. This work was done in collaboration with Drs. Woods and Engle, Cornell University Medical College. The results indicated that the amino acid distribution differed significantly from patient to patient. The concept of individual specificity of these proteins is being further tested. Parts of this work have been reported at various national and local meetings. Publications on these subjects are in press and in preparation.

(b) SEROLOGICAL AND IMMUNOLOGICAL APPROACH:

Reports appearing in the literature have suggested the possible role of sulfated mucopolysaccharides as the agents giving rise to production of rheumatoid factor. Careful immunological and serological investigations now show these suggestions to be devoid of merit. (Report on this in press.)

The role of immune complexes (antigen-antibody) in the production of rheumatoid factor was investigated by in vitro studies. Rheumatoid factor was shown to have a high affinity for certain antigen-antibody complexes, particularly if the system contained human antibody or if the antigenic material was a molecular aggregate of human gamma globulin. Preliminary findings will be reported by Dr. Josue Corcos at the annual meeting of the American Rheumatism Association.

(c) THE CELLULAR ORIGIN OF THE RHEUMATOID FACTOR:

Collaborative work with Drs. Mellors and Korngold resulted in the development of a reagent for the detection of rheumatoid factor in tissues. The work has been published, and further investigative work is in progress. (See report by Mellors, Korngold, et al.)

(2) *Other Connective Tissue Diseases*

The occurrence of auto-antibodies in Systemic Lupus Erythematosis, scleroderma and dermatomyositis was investigated. Methods, including complement fixation and agar gel diffusions, were developed for the demonstration of antibodies in these diseases, which seem to be directed against cell nuclei or constituents thereof. A selected clinic population is being screened for such complement fixing antibodies.

Laboratory of Analytical Biochemistry – Dr. C. J. Umberger

Experimental work carried out in 1959 confirmed earlier observations which indicated that N-methyl pyridine derivatives were normal urinary metabolites. Two closely related compounds have been identified in the urinary fraction that give the typical ultraviolet absorption for N-methylated pyridines. Trigonelline was isolated from pooled post-infectious hepatitis urine in quantities sufficient to permit establishing the structure from carbon, hydrogen, nitrogen, and molecular weight determinations. N-methyl pyridine chloride, which was first thought to be an artifact from the chemical treatment in isolating the methylated pyridine, was shown to be a normal urinary component by chromatographic absorption on very long columns. Studies with continuous collection of individual urinary samples from normal subjects over several days showed that the excretion patterns for these substances closely paralleled uric acid output. The output curve also fluctuated in the same direction with creatinine output, but the quantitative correlation was not nearly as close as for the uric acid excretion. This study suggests that the N-methyl pyridine compounds are involved in body metabolism and possibly arise from degradation associated with muscle changes, which have been shown to involve substances with the pyridine nucleus. Further investigation in diseases such as myositis fibrosa, myasthenia gravis, and muscular dystrophy are indicated. Immediate plans include a survey of the urinary excretion in acute gout.

The presence of N-methyl pyridines in urine, reported as early as 1912, was later discredited when it was found that these substances were present in coffee. An extensive investigation during 1959 into the possible role of coffee as the source showed that between 30-50 cups per day would have had to be consumed in order to account for

the normal urinary excretion. Control experiments on laboratory personnel during high coffee ingestion failed to show any significant change in urinary methyl pyridine output. Analysis of a number of brands of instant coffee showed that between 60 and 80 percent of the initial pyridine content of the bean was destroyed during treatment to prepare the instant brand. It was also found that, instead of the original pyridine products, the pyridine component present in the treated product was methyl pyridine hydroxide, a compound which produces hypertension. This was observed in the highest concentrations in the so-called "caffeine free" brands.

One of the major difficulties arising from the transfer of our Laboratory from the Hospital for Special Surgery in 1958 to the "Kips Bay Laboratory" was the lack of patient material and the problem of coordinating laboratory findings with the clinical follow-up. The myositis ossificans patient, was followed throughout her period of steroid treatment. It was found that N-methyl pyridine excretion dropped and then went down to zero output on cortisone. The initial pretreatment level did not return within the period of study, after cortisone was stopped. The uric acid and creatinine outputs were also considerably lowered on cortisone. Evidence showing that the lowered and negative methyl pyridine concentration was a cortisone effect was obtained by a number of urinary studies on rheumatic patients on steroid therapy. Creatinine, which was determined in these patients in order to evaluate urine collection, was also found to be low and variable. This indicated that the creatinine content of the urine is not a reliable index for urinary output for patients under steroid therapy.

In previous work, a very high N-methyl pyridine excretion had been observed in infectious hepatitis and in early cirrhosis. During 1959, four patients with jaundice from extra-hepatic blocks were studied. All of these showed high transaminase levels as found in hepatitis but the N-methyl pyridine content was not as high as the average for normals in two of these patients and was nearly zero in the other two cases. This finding was further confirmed in a recent patient transferred to New York Hospital for surgery who was found to have excessive stone formation in the bile ducts. It suggests a direct diagnostic application to differentiate between intra- and extra-hepatic jaundice and is also of interest with regard to the role of liver function in stress because of the negative N-methyl pyridine content of the urine in the immediate post-operative period.

Considerable work was accomplished in 1959 toward improving and developing analytical methods for evaluating urinary products. The binding of urea and the interferences which binding exerts on analytical determinations were further demonstrated by chromatography experiments on specially treated paper and by studies of dialysis rate through graded membranes.

Robert C. Mellors, M.D., PH. D.

Etiology of Cancer and Connective Tissue Diseases

Study of Cause and Characteristics of Cancer:

Cancer is an autonomous new growth of tissue which is perpetuated by an endless succession of cell divisions. There are two principle concepts concerning its causation:

1. Cancer may be caused by some unknown, internal factor which interferes with the chemical machinery that ordinarily regulates or controls cell division; and

2. Cancer may be caused by the entrance into the cell of some extrinsic agent which interferes with the regulatory chemical machinery. Such extrinsic agents in the form of viruses are known to cause cancers of certain types in some experimental animals (chickens, rabbits, mice, hamsters). The viruses are nucleoproteins and in this respect bear a chemical similarity to the self-replicating constituents of the organism (chromosomes, genes, nucleoli, microsomes) which regulate cell division and cell growth. In fact upon entrance into a cell, a virus may become integrated into this cellular machinery and there mediate its effect, acting as though an intrinsic part of the cell. Thus integrated the virus is no longer recoverable from the cell; and the concepts of cancer causation by intrinsic and extrinsic agents thus appear to merge as one. We have used the fluorescent-antibody method to study the cellular sites of origin and development of viruses in the experimental cancers which they produce. This affords an opportunity, not yet achieved in human cancer studies, of analyzing the development and growth of a cancer while at the same time identifying the causative agent (or its associated antigens). By this means it is hoped to acquire better insight into the cause of the generality of cancers, including those affecting man.

Pathologic and Immunologic Studies of Connective-Tissue Diseases

Rheumatoid arthritis is the most common form of progressive debilitating joint disease. The first pathological change occurs in the synovial membrane, the specialized lining of the joint capsule which plays an important role in the nourishment of the articular cartilage. For reasons unknown, in rheumatoid arthritis the synovial membrane becomes inflamed and spreads over, destroys, and replaces the articular cartilage. Microscopic examination of biopsies of the synovial membrane indicates that there are many inflammatory cells in the inflamed membrane and that among these are plasma cells which in other locations in the body and under other circumstances are known to produce antibodies or immune bodies, such as those that combat bacterial infection. But in rheumatoid arthritis there is no known bacterial infection of the joints so that the presence of plasma cells presumably has some other significance. Since it is known that antibody-like proteins called the rheumatoid factor are present in the blood serum of the majority of patients with rheumatoid arthritis, it seems possible that synovial plasma cells are the source of rheumatoid factor, as suggested in our progress report of the previous year. That this is true has been fully illustrated by staining tissue biopsies with a newly developed dye labeled reactant for rheumatoid factor. Two categories of cells are found to form rheumatoid factor: plasma cells in the synovial membrane, and large pale cells in lymph nodes. While continuing and extending this initial study of the cellular origin of rheumatoid factor we shall endeavor to determine why it is formed and thus hope to gain knowledge of the cause of rheumatoid arthritis. (This work, which was carried on with the collaborative efforts of Doctors Heimer and Korngold of our Research Laboratories, was presented at the meeting of the American Rheumatism Asso-

ciation in December in Detroit and has since been published. It represents a real "break through" in proving the cellular origin of the rheumatoid factor, formerly found only in the patient's blood serum. PDW)

Dr. L. Korngold

Determination of specific antigens of tumor tissues

Until moving into the laboratory of the Caspary Research Building, it was possible to do a very limited amount of work at the Sloan-Kettering Institute for Cancer Research. This work consisted primarily of the standardization of methods to be applied at the Hospital for Special Surgery. These methods included immuno-electrophoresis; the fractionation of serum proteins by ion-exchange column chromatography and the development of new methods of immunological analysis.

The possibilities and limitations of the double gel diffusion method were explored and several new applications of the system were developed. This method was then applied to the study of the "abnormal" serum proteins of patients with multiple myeloma and macroglobulinemia. The antigenic properties of these proteins which were formed by the plasma cells, have been described, as were the antigenic relationship of these serum proteins to the urinary Bence Jones proteins eliminated by many of these patients. Our studies have resulted in sensitive immunological tests for multiple myeloma and macroglobulinemia.

Antisera were prepared against human tissues and tumors and the distribution of several tissue antigens in many tissues and tumors obtained from surgery was established with these antisera. Moreover, they were useful in keeping track of transplantable human tumors grown in cortisonized animals. The specificity of some tumor antigens was suggested. A more thorough study of the antigens of human leukocytes and human cells grown in tissue culture has subsequently been initiated. The results show that granulocytes contain specific antigens as do some of the cell lines grown in tissue culture.

The objectives for the immediate future are:

1. To establish whether normal human gamma globulin is antigenically heterogeneous, and if so, whether this heterogeneity can explain the specific properties of the "abnormal" serum proteins from patients with multiple myeloma.

2. To develop a qualitative immunological test for Waldenstrom's Macroglobulinemia and to define the abnormal features of the patients' macroglobulins.

3. To continue with the attempts to find more specific leukocyte antigens, especially for the lymphocytes.

James C. Harkin, M.D. — *Electron Microscopic Laboratory*

From July to December, 1959, the electron microscopy laboratory in the Caspary Research building was still in course of construction and no work could be done. Nevertheless, several accomplishments by this section may be enumerated:

1. Consumable supplies and supplemental equipment were purchased for the new laboratory. This work was aided by the research assistant, Miss Aina Kraujitas. A grant of $12,000 per year from the U. S. Public Health Service, H-4841, made to the chief investigator, helped support this research work after activation September 1, 1959.

2. New work was started on a limited scale with the cooperation of the staff at the Sloan-Kettering Institute, especially Dr. Fred Shipkey. Tissues were prepared for later examination by light and electron microscopy.

3. Several projects were completed where electron micrographs had been taken earlier. Published was a study made with Dr. Lillian Recant, Washington University:

Harkin, J. C., and Recant, L.: Pathogenesis of experimental nephrosis. Electron microscopic observations. Am. J. Path. 36:303-330, 1960.

Material was organized for a demonstration that will be presented at the meeting of the American Association of Anatomists in New York City, April, 1960. The title is: Electron microscopic observations of kidney. Ultrastructural parallels of certain functional variations. Two manuscripts have been completed to the point that they will soon be submitted for publication; one covers ultrastructure of renal embryology and the other fine structure of prostatic cells studied at various ages. A number of additional electron micrographs were given to Dr. Dorothy Price, The University of Chicago, for her use in a new text on embryology.

Dr. Fred Rapp — *Laboratory of Micro-biology and Virus Research*

The virus laboratory has been organized primarily for basic studies concerned with cancer and crippling diseases. It is now known that viruses are capable of causing various types of cancer in many species of animals; many investigators believe that they may also be responsible for a number of human cancers.

Such agents (viruses) can multiply only in living cells; in doing so, they may destroy the cell, leave it relatively unharmed, or transform a normal cell into one having the properties of a cancer cell. This transformation can take place *in vivo* (in the body) or *in vitro* (in tissue cultures which consist of cells growing in test tubes or bottles). It is planned to study the conditions required for such transformations to occur as well as to establish criteria for the detection and recognition of viruses capable of causing tumor formation. Such investigations are prerequisites for a study of the virus etiology of human cancer; human cancers will be studied as soon as some of these criteria have been established in this and other laboratories.

The laboratory has therefore been designed so that potentially hazardous agents can be handled with a minimum of risk. Such agents will be manipulated in the two small sterile rooms by personnel especially trained in the proper procedures to be employed. Equipment for decontamination and special facilities required to insure growth of tissue culture cells are also available.

Tissue cultures will therefore be extensively utilized. It is also planned to use them to study many aspects of rheumatoid diseases. Work of this nature will often be performed

in collaboration with other investigators. It is planned to employ immunological methods (such as the fluorescent antibody technic and neutralization and complement fixation tests) in both virus studies and studies concerned with other problems of special interest to the staff of the Hospital for Special Surgery.

Felix Bronner, Ph.D. — *Laboratory of Mineral Metabolism*

Work in 1959 centered on five areas: *a.* completion of studies dealing with the renal handling of electrolytes in dogs; *b.* initiation of work on the effect of diethyl-stilbesterol on the mineral metabolism of human beings; *c.* initiation of studies on the effect of immobilization on calcium and strontium metabolism of adolescents; *d.* calcium absorption studies done on an outpatient basis in patients with and without osteoporosis; *e.* ion flux studies in smooth muscle of rabbits.

a. *Studies in Renal Physiology:*

In collaboration with Dr. D. D. Thompson, Department of Medicine, Cornell University Medical College, it was demonstrated that calcium, sodium, strontium and potassium in the urine of anesthetized dogs subjected to mannitol diuresis derive both from ions filtered at the glomerulus and from ions that have crossed the tubular cells from the blood into the urine as it forms in the tubule. Although our evidence is consistent with the possibility that secretion occurs for calcium, sodium and strontium — that most of the urinary potassium originates by secretion is now well established — the phenomenon observed by us is described more appropriately by the term "transtubular flux." In the case of magnesium and phosphate, on the other hand, the transtubular component was negligible under our experimental conditions. Because it was believed until recently that most of the ions in the urine are derived only from ions filtered at the glomerulus, this work has contributed to a better understanding of the mechanisms by which the urine is formed.

b. *Effects of Stilbesterol on Calcium Metabolism*

(Project No. 3712)

Postmenopausal osteoporosis is commonly treated by the administration of estrogens and/or androgens, but the number of objective studies on which this therapy is based is small. By combining balance with isotope studies, it is hoped to obtain information on how stilbesterol (a synthetic estrogenic substance) affects calcium metabolism.

Two healthy women, one premenopausal (41 years old) and the other postmenopausal (59 years old) were studied during the past year. It was found that in the older patient the administration of stilbesterol caused the urinary output of calcium to drop by 41%. Following cessation of therapy, her calcium output rose slowly, but had not yet reached the control level four weeks later when the study was stopped. The drug had little effect, however, on the urinary excretion of phosphorus, nitrogen or creatinine.

The drop in urinary calcium output was not paralleled by a similar drop in Ca-45

output (the tracer had been given by intravenous injection during the control periods and while treatment was under way). This is interpreted as evidence that stilbesterol administration diminished the rate of bone resorption.

In the younger (premenopausal) woman, estrogen administration had no effect on the urinary electrolytes or on the excretion of Ca-45. However, after cessation of stilbesterol therapy, her urinary excretion of calcium rose and remained elevated for the remaining four weeks of study, without there having occurred a corresponding rise in Ca-45 excretion. This is interpreted to mean that endogenous estrogenic activity had been suppressed by the drug and had not returned to normal during the post-treatment observation period.

These studies provide important evidence on the effect of stilbesterol on the calcium metabolism of human beings. When buttressed by further work now in progress, they may add to the rationale of the hormonal treatment of postmenopausal osteoporosis.
(Supported by a grant from the National Institutes of Health.)
(In collaboration with Drs. Paul D. Saville and James S. Nicholas.)

c. *Calcium and Strontium Metabolism in Immobilized Patients*

(Project No. 3711)

When patients are immobilized in plaster of Paris jackets, their urinary calcium output will rise. The origin of this calcium is unknown, *i.e.* whether resulting from a relative or absolute increase in bone resorption and/or a decrease in bone formation. The purpose of these studies is to obtain data that might help shed light on these questions.

Until recently, the metabolic behavior of strontium had been considered to be similar to that of calcium. Although the dissimilarity in physiological behavior of these two minerals is being increasingly appreciated, it seemed of interest to study possible differences between calcium and strontium so far as bone metabolism is concerned.

To these ends, patients scheduled to undergo surgical correction for scoliosis were admitted to the metabolic ward and studied while still ambulatory and later, after they had been placed in the cast. Just before they were to be operated on, they were discharged from the study, but readmitted for study after having convalesced for several weeks.

Studies on two patients have now been completed. In one patient immobilization in a cast was followed by a rise in calcium output, but a drop in Ca-45 excretion. This is interpreted as reflecting a rise in bone resorption. Two months later, after the patient had convalesced from the operation, but while still in the cast, his increased urinary calcium output persisted, but now he excreted a larger than normal fraction of the radioactive calcium. This is interpreted as resulting from a decrease in bone formation.

Interestingly enough, there were no corresponding changes in the excretion of radioactive strontium, suggesting that in this patient, the profound effects of immobilization on calcium metabolism were not reflected in strontium metabolism.

In the second patient, the rise in urinary calcium excretion was more marked during the first phase of immobilization, but did not persist after convalescence from the

operation. However, there was a corresponding change in the Ca-45 excretion, so that it is not possible to specify possible changes in bone formation or resorption rates.

In this patient, too, the rise in calcium and Ca-45 excretion was not accompanied by a corresponding rise in Sr-85 excretion. Here again, the effects of immobilization on calcium metabolism were not reflected in strontium metabolism.

These studies, now being actively continued, reveal useful information on the metabolic responses of the skeleton and, if confirmed by further work, may contribute to a better understanding of the differences in the physiological behavior of calcium and strontium, a topic of considerable interest in this nuclear age.

(Supported by a grant from the Atomic Energy Commission.)

(In collaboration with Drs. John R. Cobb, James A. Nicholas, Paul D. Saville and Philip D. Wilson, Jr.)

d. *Calcium Absorption Studies*

A technique has been developed to study calcium absorption on an outpatient basis. So far 28 studies have been completed on 23 patients. It was found that calcium absorption in older people is generally appreciably lower than absorption in younger individuals. Because of the wide variation from individual to individual, it is not yet known how and whether calcium absorption in normal persons differs from absorption in persons with known bone disease, but this question is now under active investigation.

We have been fortunate in securing a grant from the National Dairy Council to support these studies in 1960.

(In collaboration with Drs. Paul D. Saville and James A. Nicholas.)

e. *Ion Flux Studies in Smooth Muscle*

In collaboration with Dr. C. Y. Kao, Downstate Medical Center, Brooklyn, N. Y., studies were initiated to determine whether and how the behavior of sodium and chloride ions in smooth muscle differs from that in skeletal muscle. To this end strips were prepared from the uterus muscle of estrogen-treated rabbits and studied in radioactive salt solutions. Even though the chemical composition and electrical behavior of the uterine (*i.e.* smooth) muscle differ widely from those of skeletal muscle, the behavior of Na and Cl ions was not very different from their known behavior in skeletal muscle. It was found that electrical stimulation of the smooth muscle raised its content of radioactive sodium ten times and that of radioactive chlorine twice. These results also indicate that uterine muscle is more permanent to Na than to Cl and may contribute to an understanding of the role of these ions in the contraction of this poorly understood, but important, tissue.

During the year this laboratory, along with the others of the Research Department, moved to new and spacious quarters in the Caspary Research Building. The staff has now grown to five technicians, a dietitian, one laboratory and one diet aide, a secretary and a computational assistant (part-time).

In the course of the year I have attended several scientific meetings, including the International Congress of Physiological Sciences held in Buenos Aires, was Chairman

of one of the sessions of the Gordon Research Conference on Chemistry, Physiology and Structure of Bones and Teeth, and participated in a Workshop on Bone Densitometry that had been convened at the National Institutes of Health in Bethesda. Papers were delivered at meetings of the Federation of American Societies for Experimental Biology and of the American College of Surgeons. I was elected to membership in the American Physiological Society and in the American Federation for Clinical Research.

Two publications have appeared from this laboratory during 1959:

a. Bronner, F., Some Aspects of the Metabolism of Bone Salt.
Bull. Hosp. for Spec. Surg. *2*:44, 1959.

b. Bronner, F. and D. D. Thompson, Urinary Excretion of Calcium in Anesthetized Dogs.
Fed. Proc. *18*:17, 1959.

Paul D. Saville, M.D. — *N.I.H. Grant A-3573 A*

180 biopsy specimens were taken from 140 cadavers in the Chief Examiner's mortuary, Bellevue Hospital. Fat-free dry weights were measured and grouped in decades in the two sexes.

The changes in bone density with age and sex were noted and discussed at Research Seminar, Hospital for Special Surgery, and at a Workshop for Bone Densitometry, Bethesda, Maryland. The work supported by this grant has now been completed and is *in preparation for publication.*

Cerebral Palsy Clinic — Dr. William Cooper

Projects No. 3760 G, No. 3761 F

1. A study of the end results of surgery in over 200 patients with cerebral palsy will be completed for presentation to the American Academy of Cerebral Palsy in October, 1960, with Dr. Wm. Arnold.
(Supported by U.C.P.A.)

2. Data analysis cards are being kept current on all patients in Cerebral Palsy Clinic. These have permitted numerous statistical studies of various aspects of cerebral palsy.

3. Study on diagnostic inaccuracy in cerebral palsy (with Dr. Tambakis). This will include a review of known errors in diagnosis in 1000 consecutive cases from the cerebral palsy clinic.

4. Three 20 minute moving picture sequences will be prepared in Cerebral Palsy Clinic during 1960 on:

1. Diagnosis of cerebral palsy
2. Treatment of cerebral palsy
3. Life history of the person with cerebral palsy.
(Partial support by U.C.P.A.)

J. Paul Harvey, Jr., M.D. — *Study of Water Soluble Media (Contrast U) for Myelography*
Project No. 3302 F

With the assistance of Dr. Warner from the Pharmacology Department of the Cornell Medical School, experimentation has continued on exposed spinal cords of cats.

It was demonstrated that the material used causes a loss of conduction during the time that it is applied to the nerve root; then, upon removal of the material normal conductivity returns.

An exhibit was shown in Chicago with demonstration of myelograms and the clinical results. Excellent response was obtained and since then many letters have been received requesting information on this material.

We plan to continue experiments in conjunction with the Pharmacology Department and the Neurological Department of this Hospital.

Konstantin P. Veliskakis, M.D. — *Study of Round Back (Kypho-scoliosis)*
Project No. 3002 F

This clinical study of patients with round back deformity that have attended the Scoliosis Clinic in the past 25 years was begun in September, 1959. Part of the study was collection of material from the records of these patients to determine the clinical aspects of patients with this condition with particular emphasis on the long-term follow-up and the results of treatment.

A preliminary report on this study was given at the Resident's Conference in October, 1959. This included a report on the growth of the normal vertebral body, development of physiologic vertebral column curves, the ortho-mechanics of the spine and concepts of pathogenesis of adolescent kyphosis.

The number of cases studied so far is 129 and the investigation is still underway.

The number of patients that have attended the Scoliosis Clinic in this period of time is about 500. Each patient is written to and asked to report here for a clinical examination and possibly X-rays.

Because of the nature of the condition, the majority of these patients are not on the active list of the Scoliosis Clinic and, since in general they are asymptomatic, it is not easy to get them back for re-examination.

DELAYED PROJECTS

Long Term Follow-up Study of Rheumatoid Arthritis —
Drs. R. Cecil and Wm. Kammerer — Project 3102 F

This study, designed to obtain knowledge of the course of the disease in patients who have suffered from it for many years and who have been attending the Arthritis Clinic of the Hospital, has now been in course since 1956. Over 200 cases have been studied. Conclusion of the study has been delayed in an effort to obtain autopsy reports and final

diagnoses in the patients who have died. It is hoped that difficulties can be overcome but slow progress is anticipated.

Tissue Culture Laboratory — Dr. P. Marchisello — Project 3730 F

The study of osteogenesis by tissue culture methods has been blocked temporarily by the move of research facilities to the new building and delay in completing the new laboratory. This is a temporary situation and it is hoped that the work can be renewed in the near future.

Osteosynthesis of Bone by Plastic Cement — Dr. Bernard Jacobs — Project 3301 F
Project 3301 F

This study is continuing using poly-urethane foam (Ostamer) in animals. Many lessons have had to be learned from experience and many problems have had to be solved. No conclusions have been reached but experiments are being continued using a recently prepared improved material.

COMPLETED PROJECTS

The following projects have been completed and papers are being prepared or have been published when the results obtained justified it.

Arthrogryposis Congenita Multiplex —
Dr. Victor Mayer and Associates
(23-56-0)

Epiphyseodesis and Treatment of Inequality of Leg Strength —
Drs. T. C. Thompson, L. R. Straub and Philip Granieri
(30-57-0)

Neurofibromatosis and Scoliosis —
Drs. J. R. Cobb, P. D. Wilson, Jr., and C. Veliskakis
(32-57-0)

Orthopedic Disorders of Genetic Origin —
Dr. Wm. Arnold
(40-58-0)

Induction of Bone Formation in Rats —
Dr. John B. Sullivan
(49-58-0)

Experiments were conducted on rats using acid pepsin digested tracheal cartilage from the beef and auto-transplants from the renal pelvis epithelium. Negative results were obtained and the experiments were terminated.

FINANCIAL

An audited statement of the financial operations for the year 1959 showing changes in Education and Research fund principal is published elsewhere in this report.

As 1959 developed, our occupancy of the new research facility was delayed. It was not until early December that the first of our new laboratories came into operation. Consequently, the full impact of our expanded research program did not manifest itself in 1959, as had been anticipated. In terms of total intake, we enjoyed a favorable result. Our total additions to working capital amounted to approximately $364,000.00 and our reductions therefrom totaled $314,000.00. The resultant increase of $50,000.00 reflects additions to U.S.P.H. grant capital for research projects and also additions to special-purpose fund capital available only for restricted use.

Significantly, our expenditures for institutionally supported activity increased to approximately $196,000.00 and institutional fund support leveled out at approximately $189,000.00. While the operating deficit for 1959 was a relatively modest $7,000.00, our anxiety expressed prematurely in 1959 appears to be coming to maturity in fiscal 1960. Since November we have occupied our newly completed laboratory facilities and have gotten under way with an enlarged research program. We foresee total expenditures amounting at least to $340,000.00 per year. When compared to income of approximately $190,000.00 in 1959, the need for an additional $150,000.00 per year of unrestricted support becomes only too apparent.

At the close of 1959 we had expended approximately $2,400,000.00 for construction and scientific equipment. As an offset we had building funds principal amounting to $2,477,000.00. At the time, our investment represented a level of completion equal to 87% of our needs as now conceived. The most recent projection of our building budget indicates a cost of $2,863,000.00 for construction and for scientific equipment; or a cost of $385,000.00 in excess of building fund capital at year's end. To offset this imbalance the Philip D. Wilson Research Foundation offered to advance $350,000.00, thereby leaving a foreseeable building fund deficit of approximately $35,000.00 as of this writing.

Respectfully submitted,
PHILIP D. WILSON, M.D.
Director of Research

The new Alfred H. Caspary Research Building.

Main entrance to the Research Building.

DEPARTMENT OF EDUCATION AND RESEARCH
PHILIP D. WILSON RESEARCH FOUNDATION

Details of Changes in the Research Funds
During the Year Ended December 31, 1958

	Total All Funds	*Education and Research Funds*	*U. S. Public Health Grants*	*Atomic Energy Commission Grants*	*Other Grants*
BALANCE AVAILABLE AT DECEMBER 31, 1958.....	$254,805.11	$185,778.09	$28,010.88	$10,294.04	$30,722.10
ADDITIONS:					
Income earned and appropriated	49,469.64	49,469.64	—	—	—
Gifts and Grants received	315,183.58	140,055.72	109,779.00	18,831.00	46,517.86
TOTAL ADDITIONS ...	364,653.22	189,525.36	109,779.00	18,831.00	46,517.86
EXPENDITURES:					
Salaries	184,947.05	124,764.62	40,193.51	10,359.74	9,629.18
Expenses	66,277.30	36,489.22	18,736.67	5,408.98	5,642.43
Overhead	36,000.00	22,253.21	7,943.75	4,382.18	1,420.86
Equipment Purchased....	27,639.52	12,681.45	5,057.93	400.00	9,500.14
TOTAL EXPENDITURES	314,863.87	196,188.50	71,931.86	20,550.90	26,192.61
BALANCE AVAILABLE AT DECEMBER 31, 1959.....	$304,594.46	$179,114.95	$65,858.02	$ 8,574.14	$51,047.35
DECEMBER 31, 1958.....	$254,805.11	$185,778.09	$28,010.88	$10,294.04	$30,772.10

Department of Education & Research

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